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Investigating movement in the laboratory: dispersal apparatus designs and the red flour beetle, *Tribolium castaneum*

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Abstract

The natural dispersal of *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) has been emulated in the laboratory for more than 50 years, using a simple dispersal apparatus. This has typically comprised of a starting container (initial resource or patch) connected by tubing, which contains thread for the animals to climb into a tube and hence to an end container. That is, beetles move to a new viable resource or patch from an inter-patch zone or non-viable habitat. We modified this basic apparatus design to test the effect of tubing length and tubing insertion angle on the dispersal rate and proportion of successful dispersers. We expected that the proportion of successful dispersers would be repeatable within each apparatus design, and that increasing tubing length and steepness of the insertion angle would reduce dispersal rate and success across apparatus designs. Dispersal increased linearly through time, similarly so for both males and females. The design with the most vertical tubing insertion angle had a lower proportion of successful dispersers. Tubing length also had a negative relationship with dispersal success (as judged by insects reaching the end container), but a significant reduction in dispersal success was only apparent between the shortest and longest tubing between containers. We suggest that locating and climbing the vertical section of string before they can enter the tubing between containers restricts dispersal and that at higher densities, insects exhibit greater inclination to climb. This type of apparatus has flexible design tolerances and further potential to study the dispersal of other small insect species that primarily use pedestrian locomotion.

Introduction

Flour beetles of the genus *Tribolium* (Coleoptera: Tenebrionidae), particularly *Tribolium castaneum* Herbst and *Tribolium confusum* Duval, are major pests of a wide range of grain species and processed stored products globally, and are known to have a high rate of movement among resource patches (Campbell & Hagstrum, 2002; Ahmad et al., 2012). A diverse array of approaches have been used to study the movement of *Tribolium* beetles including laboratory apparatuses (Prus, 1963; Łomnicki, 2006), warehouse-level patch exploitation arenas (Campbell & Hagstrum, 2002; Campbell & Arbogast, 2004), and landscape-scale sampling (Ridley et al., 2011). Here, we focus on movement in apparatuses. Most apparatus designs consist of connected containers that adult beetles can move between, allowing dispersers and non-dispersers to be separated over time. The main phases of successful dispersal can be observed with this design: the inclination to move away from a resource patch (initial container), survival through an unsuitable inter-patch zone (tubing between patches, or in intermediate containers of various suitabilities), and establishment into a new patch (final container) (Bowler & Benton, 2009). Although this has been the most widely used approach, little consensus has emerged on certain apparatus design attributes.

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Prus (1963) described an apparatus to study the tendency for beetles to emigrate from a vial containing flour (A) to an empty vial (B) by climbing cotton thread into a tube. The tubing was inserted through the vial lids (bridging the two vials) and the thread dangled onto the surface in A but not in B, permitting only one-way movement from A to B. Emigration rate of beetles from A to B was repeatable over 10 days and multiple experimental replicates (Prus, 1963). Despite inexplicit design specifications (4.5 mm tubing internal diameter; initial density: 32 beetles per 8 g of flour), the repeatability of the recorded behaviour and design simplicity ensured its longevity in subsequent studies of *Tribolium* dispersal. Modifications to the Prus design such as that of Ogden (1970a,b) included flour medium in the second vial, and also defining the length, shape, and material of tubing (Tygon tubing, U shaped, and 150 mm long), container size (29.5 ml), and population density (either 32 or 50 individuals per 8 g of flour). Ziegler (1976) utilized a pipe cleaner, in place of thread, and glass tubing, but described no other design parameters.

The Prus design was used as a platform for investigating *Tribolium* emigration in response to numerous factors, including population density, age, artificial selection for activity or emigration, and also fecundity and life-history responses to selection for emigration (Prus, 1966; Zyromska-Rudzka, 1966a,b; Ogden, 1970a,b; Ziegler, 1976, 1977; Ritte & Lavie, 1977; Lavie & Ritte, 1978, 1980; Riddle & Dawson, 1983; Zirkle et al., 1988; Goodnight, 1989; Ben-Shlomo et al., 1991). Ritte & Lavie (1977) induced divergent selection on dispersal in just one generation by selecting for beetles that moved from vial A to B (via a 30-cm-long polyvinyl tube), twice in two opportunities, as high dispersers and those that did not move as low dispersers. Łomnicki (2006) used a one-way dispersal apparatus including five beakers of various size (A–E), three of which contained flour (A, C, E), and two of which were unsuitable habitats (i.e., empty: B, D), all connected by glass tubing containing string. Dispersal through this apparatus was far slower than in previous designs, suggesting that the extra beakers limited successful dispersal rate.

Despite the differences among designs, many of these studies reached similar conclusions. Emigration tendency was low in immature individuals, peaked around sexual maturity, and declined later in life. Males emigrated more rapidly than females when the sexes were kept separately, and keeping the sexes mixed resulted in an overall emigration rate that was intermediate between those of the sexes separately (Prus, 1963; Ogden, 1970b; Ziegler, 1976; Riddle & Dawson, 1983). Dispersal was dependent on density and age of infestation; this was thought to be a response of repulsion to flour that was ‘conditioned’ by chemical secretions and frass accumulation over time (Zyromska-Rudzka, 1966a; Ogden, 1970b). A discernible difference across the emigration rates of lines selected for dispersive and non-dispersive behaviour was identified after five generations of selection (Ogden, 1970a) and dispersal behaviour has an underlying genetic component (Ritte & Lavie, 1977; Lavie & Ritte, 1978; Riddle & Dawson, 1983).

Laboratory-based studies on *Tribolium* dispersal have frequently used the apparatus design of Prus (1963) with modifications to the container size, tubing length and material, container arrangement, population density, and time period. However, many studies have not provided detailed specifications of apparatus components. The length of tubing between containers (which represents the inter-patch dispersal component), the angle at which the tubing is inserted into container lids (which may increase dispersal difficulty) are unstated, or vary significantly among studies. The importance of these factors to dispersal success has not been investigated previously. In the present study we have implemented and tested design aspects derived from apparatus revisions by addressing the following three questions. (1) Does increasing tubing length affect the proportion of successful dispersers and the dispersal rate? (2) Does tubing insertion angle affect the proportion of successful dispersers? (3) Is dispersal rate repeatable within an apparatus design? We predicted that longer tubing and more vertical tubing insertion angle would reduce the proportion of successful dispersers. We addressed these questions using *T. castaneum* and five dispersal apparatus designs, manipulating tubing insertion angle and length.

Materials and methods

Animals and housing

A wild-type population of *T. castaneum* (QTC4) was sourced from the Postharvest Grain Protection Team (Department of Agriculture, Fisheries and Forestry, Brisbane, QLD, Australia) and used to establish experimental stocks. The QTC4 strain originated from a storage facility in Brisbane in 1965. It has been cultured ever since in the absence of selective pressures from the fumigant phosphine, and therefore these insects exhibit natural susceptibility to phosphine. Stocks were maintained on 210 g of flour medium containing wholemeal stoneground wheat flour (Kialla Purefoods, Greenmount, QLD, Australia) and torula yeast (Lotus Foods, Cheltenham, VIC, Australia) at a ratio of 19:1 in 1-l cylindrical containers. Stocks were maintained in a controlled temperature room at 29.5 ± 1 °C, 40–60% r.h., and L12:D12 photoperiod. Stocks were cultured fortnightly to maintain clean housing and separate cohorts. Beetles used in experiments were collected from stocks as pupae, and sex was determined by examining the external genitalia (Halstead, 1963) under an Olympus SZ61 stereomicroscope (Olympus Australia, Notting Hill, VIC, Australia). After sorting by sex, pupae were randomly added to 70-ml containers that held 15 g of flour medium in groups of 50, such that each experimental replicate had five containers (i.e., 250 male and 250 female pupae, and a total of 3 000 individuals over six experimental replicates). They were held for 6 days to allow the resultant adults to reach 3 days of age post-eclosion before the 70-ml containers were attached as container A in the apparatus, for the dispersal experiments to commence.

Dispersal apparatus designs

Five designs were chosen to test the dispersal capacity of *T. castaneum*, based mostly on the designs of Prus (1963), Ogden (1970a), and Łomnicki (2006). Variables that were manipulated in this study were the length of the tubing and the angle of the tubing as it left and entered containers. Each design used three 70-ml containers (57 × 44 mm, labelled A, B, and C, respectively; Sarstedt Australia, Mawson Lakes, SA, Australia) connected through the lid of each container via silicone tubing (4-mm internal diameter), containing a single looped strand of cotton twine that permitted only one-way movement from container A to B to C (Figure 1).

As the angle of tubing was negatively correlated with the distance between containers, only designs 1 and 2 were compared to determine the effect of tubing insertion angle independently of length. Tubing length in design 1 was 140 mm, with a distance of 70 mm between the tube insertions, yielding a relatively steep insertion angle (55°) for the tubing to represent the extended vertical climbing distance employed in the design of Prus (1963) (Figure 1A). Design 2 used the same tubing length as design 1

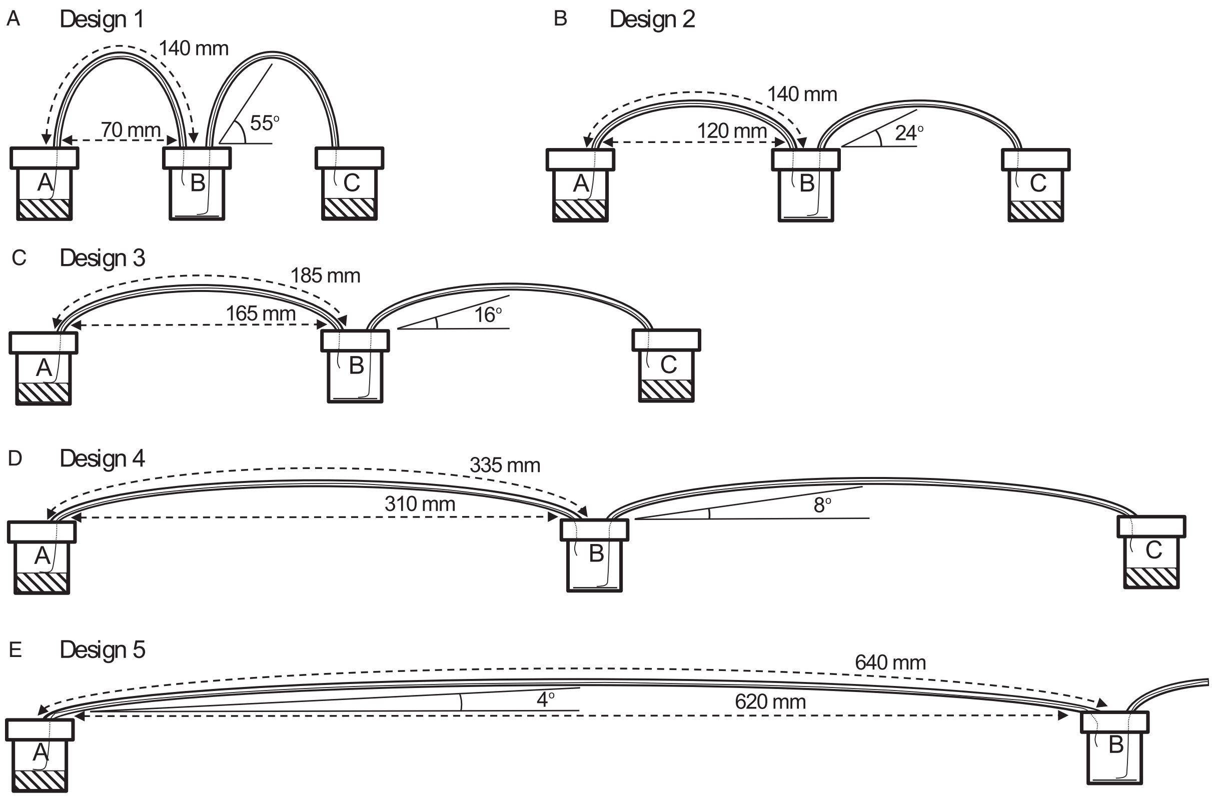

Figure 1 Dispersal apparatus designs used to investigate the effects of tubing angle and length on the dispersal success of adult *Tribolium castaneum*. Each design used three containers (A, B, C) connected by tubing containing string for beetles to climb, allowing one-way movement from A to B to C. (A) Design 1: 55° tubing insertion angle between containers over 140 mm tubing length; (B) design 2: 24° tubing insertion angle over 140 mm tubing length; (C) design 3: 185 mm tubing length; (D) design 4: 335 mm tubing length; and (E) design 5: 640 mm tubing length. In design 5, B–C is identical to A–B but not shown due to its size. Curved dashed arrows indicate tubing length, straight dashed arrows show distance between insertion points, and angles show the approximate angle from the point of insertion to the maximum height of the tubing over the distance between insertion points.

(140 mm) over a greater distance between the tube insertions (120 mm), creating a shallower slope (24°) in the tubing (Figure 1B). Designs 3, 4, and 5 all had relatively shallow tubing insertion angles (4–16°); these designs were included to test whether increasing tubing length reduced dispersal success. Design 3 used 185 mm long tubing over 165 mm (Figure 1C), design 4 used 335 mm long tubing over 310 mm (Figure 1D), and design 5 employed 640 mm long tubing over a distance of 620 mm between tubing insertions (Figure 1E). The distances between insertion points in containers in the different dispersal apparatuses were structurally maintained using plywood housing to fix the containers a set distance apart, level with each other and aligned linearly (Figure 2).

Dispersal assessment

Each design used two apparatuses, one for males and one for females, to assess dispersal for each sex separately but concurrently. Sexes were not mixed for two reasons: to eliminate the potential for breeding to occur during the dispersal process, which has been shown to slow the rate of dispersal (Ziegler, 1976) and to emulate the conditions required for a subsequent experiment that controlled breeding after the dispersal assessment. The containers with 50 adult beetles of known sex, labelled container A, were randomly assigned to an apparatus design and attached to each apparatus, representing the starting point of dispersal (Figure 2). Both A and C contained 15 g of flour and container B had a covering of paper to provide grip, but was otherwise an unsuitable habitat for the beetles. Container B was included to represent a patch that would not be a suitable resource to establish in, but that had to be passed through as part of the dispersal process (Łomnicki, 2006).

Dispersal apparatuses were placed in a controlled temperature room with identical conditions to the stock populations and apparatus position was randomized at the beginning of each experimental replicate. Once container A was connected to each of the apparatuses, dispersal assessment commenced and counts were made during 08:30–09:30 and 16:30–17:30 hours daily for 96 h (nine counts). Counts of beetles were recorded in the connecting tubes (A–B and B–C) and in containers B and C. Both sets of tubing and container B could be counted visually without disturbing the apparatus; however, container C required detachment. Flour was carefully tipped into a container and gently swept with a paintbrush to draw beetles to the flour surface for counting. Flour and beetles were then funnelled back into container C and reattached to the apparatus. Container A was left undisturbed throughout the experiment to facilitate natural dispersal through the apparatuses. The number of beetles in container A was calculated at each time point by subtracting the total number of beetles in all other containers and tubing from the starting total of 50. Mortality was assessed at the conclusion of each experiment, but was negligible (two adults at most in any given replicate). The assessment was repeated 6×, each with a different cohort of beetles, and with new apparatus materials to ensure that residues and semiochemicals did not build up in between replicates.

Statistical analysis

Data were treated as proportional data, where the principle variable of interest was mean proportion of beetles reaching container C (successful dispersers). Fixed factors were apparatus design, tubing length, insertion angle of tubing, sex, and time. Replicate (cohort) was treated as a random factor in all analyses. A full model was fitted using a generalized linear mixed-effects model with a binomial error structure and logit link function from the lme4 package (Bates et al., 2014) in R v.3.2.3 (R Development Core Team, 2015). Models were simplified by removing non-

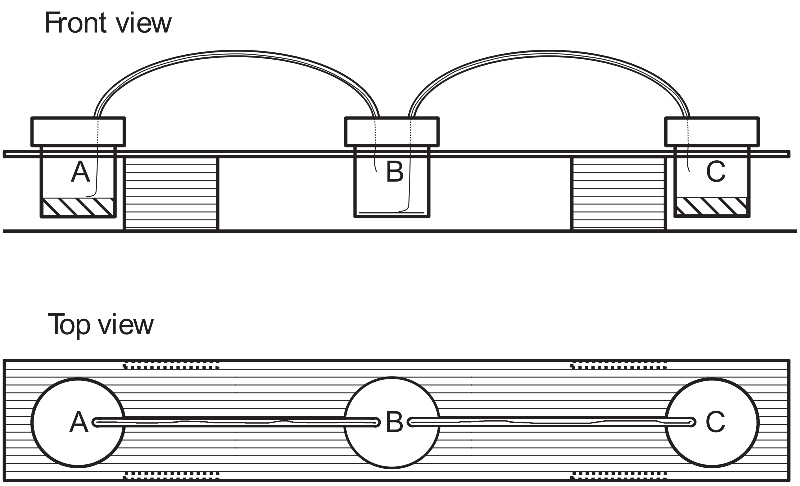

Figure 2 Example schematic of design 3. Front and top views of the plywood housing for maintaining structure and consistency of the tubing insertion angle and tubing length for the dispersal apparatuses.

significant interaction terms and using ANOVA and Akaike information criterion (AIC) to compare the resulting simplified models ($\alpha = 0.05$).

Results

Dispersal rates and success

The proportion of beetles that reached container C at any given time was relatively consistent across all designs and between sexes, where males are presented separately to females (Figure 3). The proportion of successful dispersers increased significantly with time and was repeatable, following a near-linear trajectory to about 60–80% successful dispersal after 96 h (Figure 3). Time was positively correlated with the proportion of successful dispersers and was highly significant for all apparatus designs, but no difference between male and female beetles was detected (Table 1). Design 2 was chosen as the reference apparatus against which the other designs were compared as it had the shortest tubing with a shallow insertion angle (Table 1). This design was predicted to, and did, yield the highest dispersal rates in both sexes. Only design 5 had a significantly lower proportion of successful dispersers than design 2, whereas all other designs were not significantly different from this design or from each other (Table 1).

Tube length

Increasing tubing length between containers had a negative relationship with the proportion of successful dispersers (i.e., as tubing length increased from 140 mm in design 1 to 640 mm in design 5, the regression slope decreased; Table 1). The proportion of successful dispersers was lowest in design 5, where tubing was longest between containers, and this was significantly different to design 2 (Table 1). Effectively, the greater the distance between containers, the longer it takes the beetles to move between them. Therefore, the overall proportion of beetles in container C at the end of the experiment was lower than the designs with shorter tubing between containers (Figure 3).

Table 1 Generalized linear mixed-effects model (GLMM) of the effect of time, design, and sex on the proportion of *Tribolium castaneum* beetles successfully dispersing from A to C. Designs are all compared to reference design 2 (short tubing length and shallow insertion angle)

	Estimate	SE	Z	P
Intercept	−3.788	0.187	−20.3	<0.001
Time (h)	0.555	0.001	72.11	<0.001
Design 1	−0.412	0.233	−1.76	0.078
Design 3	−0.321	0.233	−1.38	0.17
Design 4	−0.42	0.233	−1.8	0.072
Design 5	−0.565	0.234	−2.42	0.016
Sex	0.206	0.148	1.39	0.16

Random effect: replicate (intercept) variance = 0.311.

Tubing insertion angle

The angle that tubing projected from the lids of each apparatus container was predicted to have a significant effect on the proportion of successful dispersers, by increasing the difficulty of dispersal, thereby reducing the attainable dispersal rate. Designs 1 and 2 were directly comparable with respect to determining the effect of insertion angle independent to tubing length, therefore these were the only designs included in the model (Table 2). Time had a significant positive effect on the proportion of successful dispersers and males and females were not significantly different from one another, consistent with the previous

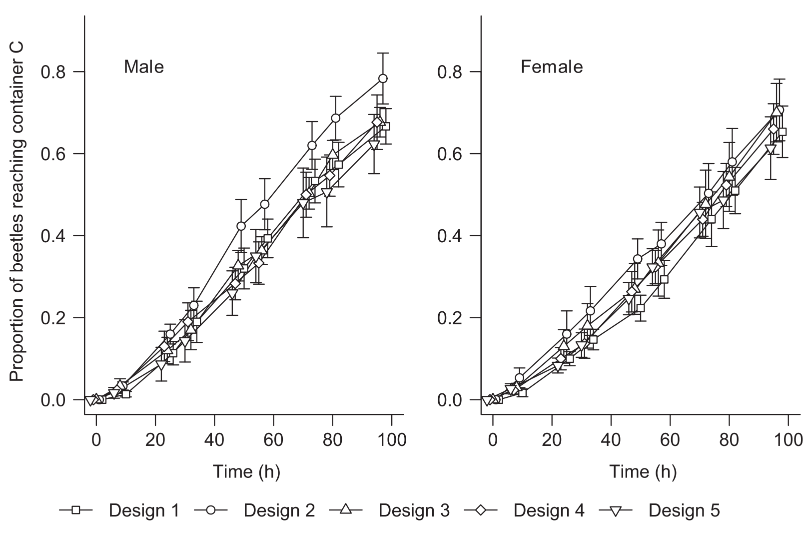

Figure 3 Mean (± SEM; n = 6 independent replicate cohorts) proportion of male and female *Tribolium castaneum* adults reaching the final container C over time across the five apparatus designs.

Table 2 Generalized linear mixed-effects model (GLMM) of the effect of time, sex, and tubing insertion angle on the proportion of successful *Tribolium castaneum* dispersers from container A to C between designs 1 and 2

	Estimate	SE	Z	P
Intercept	−3.339	0.388	−8.61	<0.001
Time (h)	0.559	0.012	46.72	<0.001
Sex	0.338	0.223	1.52	0.13
Tubing angle	−0.642	0.299	−2.15	0.032

Random effect: replicate (intercept) variance = 0.282.

model. There was a significant negative effect of tubing angle on the proportion of successful dispersers suggesting that the more vertical tubing angle in design 1 reduced dispersal success compared to design 2, which had more horizontal tubing (Table 2).

Discussion

For more than 50 years studies have used apparatuses to simulate long-distance dispersal in the laboratory using *Tribolium* beetles; however, many of these studies have not specified apparatus design attributes. In particular, the slope and length of tubing between containers were expected to be critical to the movement of the individual beetles, as even shallow increases in the slope of a gradient can affect the distribution and orientation of *Tribolium* beetles (Graham & Waterhouse, 1964). This is consistent with our finding that increasing the steepness of the tubing angle between containers reduces dispersal success. The energy required for insects to climb vertically is much greater than that required to move laterally (Full & Tullis, 1990), and as distance and time spent moving along a steep incline increases, the frequency of potential climbing errors also increases. The slope of the tubing limited dispersal across designs with identical tubing length, but the difference between the proportions of beetles that successfully dispersed, for example, in design 1 and 4, were not different. Although the effect of tubing insertion angle is significant, if tubing length is increased (i.e., to about 30 cm; Ritte & Lavie, 1977), the effect of the steep climb may be offset.

The significant effect of tubing angle suggests that the near-vertical section of string that must be climbed prior to reaching the tubing also reduces dispersal rate. The string climbing ability of *T. castaneum* has not been explicitly tested, but this species can climb paper materials (Cline & Highland, 1976), readily climbs up string within an apparatus (Ogden, 1970b), attempts to climb the walls of housing (Ghent, 1963; Surtees, 1963), and is frequently seen climbing bag stacks and walls in storages (GH Walter, pers. obs.). Across all of the designs tested in the present study, a near-vertical portion of string (about 30 mm long) came immediately before the section of tubing where the angle could then be engaged by the climbing beetle. This section requires an ability to climb successfully upwards into the tubing, which plateaus, and then descends towards the next container. It seems likely that the near-vertical climb to the tubing would also constrain dispersal rate, and this may partially explain the exceptionally slow dispersal of *T. confusum* in the study by Łomnicki (2006), as the beakers used there had a vertical string section greater than 40 mm between the flour surface and the tubing. Therefore, care needs to be taken to ensure that the near-vertical section of string is consistent in length, as it has a similar or perhaps stronger effect on dispersal rate than the tubing insertion angle. As *Tribolium* beetles are highly mobile animals, this design component is essential to constrain movement to sort dispersers from non-dispersers over a practical period of time.

Although we found an overall significant decrease in the proportion of successful dispersers with increased tubing length, this effect was only apparent when comparing the shortest (140 mm) and the longest (640 mm) tubing between containers. The long tubing used in design 5 reduced the proportion of successful dispersers but was only significantly lower than in design 2. Additionally, the extreme length between containers of design 5 made it impractical due to size. For the remaining tubing lengths between the containers (140–335 mm) dispersal rates were similar, which indicates that at least within a practical range, time spent within the apparatus tubing does not strongly limit dispersal. In contrast to our initial expectations, steepness and distance between sites did not strongly affect dispersal success. Our data therefore suggest that once an individual begins dispersing from its natal site, it will continue to disperse until it reaches another appropriate site.

In *Tribolium* beetles, dispersal is dependent on habitat deterioration, where increasing population density or reducing flour volume increases dispersal rate due to conditioning of the flour by frass accumulation, nutrient depletion, and release of quinones by adults as toxic defensive secretions (Zyromska-Rudzka, 1966a; Ogden, 1969). Adult *T. castaneum* are strongly repelled by the smell of same-sex conspecifics, and this repulsion is enhanced when flour becomes 'conditioned' (Naylor, 1961; Ghent, 1963; Ogden, 1970b). Thus, unmated individuals in a container with conspecifics will readily disperse as the flour becomes increasingly conditioned, and as finding mates becomes a priority. Therefore, the essentially linear increase in successful dispersers over time may reflect the decreasing population density as individuals emigrate and the continuous but ever-decreasing rate of flour conditioning as population density decreases.

We did not identify a significant difference in dispersal success between the sexes overall, but a greater proportion of males dispersed successfully in design 2. Males were predicted to disperse faster than females, as previous studies have found males are more active or exploratory in dispersal apparatuses (Prus, 1966; Ogden, 1970b). However, Ziegler (1976) found that dispersal rates were similar across males and females, as in the present study. We suggest that the absence of potential mates, and repulsion by the scent of same-sex individuals and conditioned flour, drove dispersal at a similar rate in both sexes.

The present study demonstrates that tubing length and tubing insertion angle, which have been inconsistent among previous studies, can alter dispersal success for this species but not to the extent that dispersers cannot be effectively sorted from non-dispersers. This general apparatus appears to have relatively flexible design tolerances, and can achieve repeatable, controlled dispersal over replicate experiments. For the logistics of assessing dispersal ability of *T. castaneum*, which is highly active, restricting dispersal rate is important. We suggest that in addition to tubing length and insertion angle, the process of locating and climbing the vertical string section, and the inclusion of an intermediate container, reduces dispersal to a practical rate. In the present study, the time taken for more than 50% of individuals to successfully disperse across apparatus designs (about 70–85 h) would be feasible to experimentally separate dispersers from non-dispersers. Future work could build upon the present study to determine dispersal rates of mixed sex populations under varying population densities and resource conditions using an apparatus design with a manageable tubing length and more gradual tubing angle (i.e., design 2 or 3). Further exploration of gradients and climbing surfaces, in addition to manipulating experimental conditions, could also provide useful insight into the condition-dependence of dispersal and the ecology of this important stored-product species. The apparatus designs tested here could also be used to assess the conditions affecting dispersal of other small insects that use pedestrian locomotion, including potential and current pest species. More than 50 years after its conception, the laboratory dispersal apparatus remains useful for assessing dispersal and addressing questions in microcosm-based species ecology.

Acknowledgements

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